cyclopentamethyleneketene (generated from α-bromocyclohexanecarboxylic acid bromide, zinc, and diglyme).

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Effect of Diazepam on Cognition *via* Pupillometry

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Received November 29, 1976, from the *School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication October 25, 1977.

Abstract □ Continuous pupillary readings in response to a random-digit cognition task were obtained for 20 male subjects. Ten subjects were given 10 mg of diazepam, and 10 subjects were given placebos. Additional pupillary curves were recorded for both groups at 1 and 2 hr and compared to the initial curve. Subjects were required to repeat the exact sequence of verbalized randomized digits as a measure of performance. The results indicated that the diazepam treatment group differed significantly from the placebo group in terms of a depressed pupillary response. Furthermore, the performance recall measure was significantly reduced in the diazepam group. The relationships were clarified by an analysis of covariance and variance.

Keyphrases □ Diazepam—effect on recall ability evaluated by pupillometry, humans □ Pupillometry—used to evaluate effect of diazepam on recall ability in humans □ Sedatives—diazepam, effect on recall ability evaluated by pupillometry in humans

Pupillometrics is the aspect of psychology that deals with pupillary alterations elicited by any stimulus other than light (1). Because the pupil of the human eye is innervated by both sympathetic and parasympathetic fibers in close association with the central nervous system, pupillometrics affords an excellent method of observing the effects of many different types of stimuli such as near vision, lid closure, nystagmus, fatigue, color contrast, hippus, psychopathic states, noise, exercise, and drugs on pupil size (2–12). Furthermore, the degree of pupillary dilation has been positively correlated with human cognition and retention. Peavler (9) described a sensitive means of generating pupillary cognition curves by presenting randomized digits verbally to subjects.

Tranquilizers, in addition to their antipsychotic effects, have been correlated with several pupillary responses in humans. The major tranquilizer chlorpromazine produced a miotic effect in relation to dosage form and temporal measures (12). Critical flicker fusion, which is partly a measure of pupillary functions, has been studied extensively (13). Two studies attempted to relate minor tranquilizers, benzodiazepines, to critical flicker fusion with different conclusions (14, 15).

Diazepam was selected for investigation for several reasons. The drug is absorbed rapidly, with peak blood levels occurring in 1–2 hr (16). The diazepam metabolite, desmethyldiazepam, peaks only after repeated doses of several days (17). Furthermore, diazepam and other benzodiazepines have been studied for their relation to motor and cognition tasks, which suggest a relation between pupillary changes and diazepam. For example, auditory reaction times, complex visual reaction times, and their corresponding error rates appear to be increased by the benzodiazepines (18–20). However, simulated car driving tests were not affected by diazepam administration (21).

The hypotheses of this project were that the oral administration of diazepam would affect the pupillary response curve obtained during the execution of a cognition task and would influence the ability of human subjects to perform successfully the task as measured by recall.

EXPERIMENTAL

Twenty male subjects, 18–28 years old, were assigned to either a control or a treatment group. They were instructed to fast for 2 hr prior to the experiment. The treatment subjects allowed their eyes to adapt to the

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Table I—Mean Number of Correct Responses

Hours	Seven-Digit Task		Nine-Digit Task	
	Diazepam	Placebo	Diazepam	Placebo
0	4.00	4.30	2.00	2.80
1	5.00	4.90	2.00	4.30
2	3.70[a]	6.00[a]	3.20	3.70

[a] t significant at $p = 0.012$.

5-footcandle room illumination level for 3 min. Each placed his head into the chinrest of the pupillometer system[1], which was illuminated by a neutral density wedge tachistoscope[2].

The tachistoscope was activated, and the subjects were instructed to focus their eyes on a single object of the display slide. The illumination of the tachistoscope was adjusted so that the initial pupil diameter measured between 4.5 and 5.5 mm. The entire adjustment procedure required approximately 2–3 additional min.

The experiment commenced when the recorder[3] was initiated. The event marker was programmed for 1-sec intervals. At the beginning of the 4th sec, a series of computer-randomized digits between zero and nine was read in monotone in 1-sec intervals. The subjects kept their eyes fixed on the slide for 20 sec/random set of numbers. The subjects could hear the ticking of the event marker, which served to indicate the beginning and end of the experimental measurement. One set of seven and one set of nine random digits were presented.

Following the 20-sec measurement period in which either the seven- or nine-digit sequence was read, the subjects were instructed to lift their heads from the chinrest and to recall the exact sequence of the numbers. The correct responses were recorded, and each subject was given a 5-mg oral dose of diazepam[4]. (All tablets were from the same lot.)

After 1 hr, the 10 treatment subjects repeated the experimental procedure and were given a second dose of 5 mg of diazepam. One hour later, they repeated the measurement procedures. The final measurement was conducted 2 hr following the initial measurement.

The 10 subjects of the control group were treated identically except that they were given two placebo capsules. The contents of 10-mg chlordiazepoxide[5] capsules were removed and replaced with lactose to serve as placebos.

RESULTS

The ability of the random-digit cognitive task to stimulate pupil dilation was substantiated. Figure 1 displays a typical response pattern, which peaked at 5.8 mm at the 11th sec of the experiment. The pupil diameter remained substantially above the beginning diameter during and following recitation of the randomized digits.

Table I displays the mean correct responses for both the seven- and nine-digit tasks. The performances of the treatment and placebo groups were significantly different at the seven-digit, 2-hr point. The diazepam group was able to recall an average of 3.7 digits, but the placebo group recalled an average of 6.0 digits. A difference in performance was not observed at the nine-digit, 2-hr point.

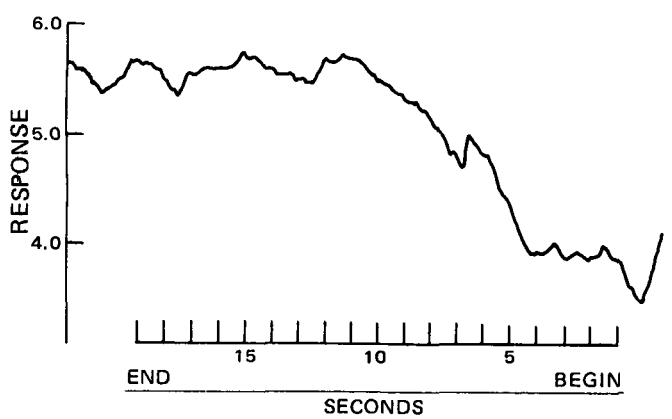

Figure 1—*Example of a pupillary response in millimeters for the 20-sec duration of the experimental observation.*

[1] Polymetric model V11651R, U.S. Testing Co., Hoboken, N.J.
[2] Model 42020, Lafayette Instrument Co., Lafayette, Ind.
[3] Model 680, Leeds and Northrup, North Wales, Pa.
[4] Valium, Roche Laboratories, Nutley, N.J.
[5] Librium, Roche Laboratories, Nutley, N.J.

Table II—Analysis of Variance and Covariance of Pupil Dilation at 11 sec of the Seven-Digit, 2-hr Task

Source of Variation	Sum of Squares	df	Mean Square	F	Significance of F
Covariate: number correct responses	0.183	1	0.183	1.245	0.280
Main effect: drug treatment	0.701	1	0.701	4.758	0.041
Residual	2.504	17	0.147		
Total	3.388	19	0.178		

The pupil dilation data were reduced to machine-readable form and treated to underscore differential treatment effects. First, the entry diameter during the 1st sec of the experiment was subtracted from the remaining seconds of observation. In this manner, differences in initial pupil sizes were removed as contaminating variables. Second, the responses during the initial seven- and nine-digit tasks were respectively subtracted second by second from the measurements of the 1st and 2nd hr. Thus, the analysis focused on measured differences in response patterns between the initial recordings and subsequent recordings of both the placebo and drug treatment groups.

Figure 2 graphically displays the difference between the placebo and treatment groups for the seven-digit task at 2 hr. The diazepam or treatment group showed a recorded value of −0.31 mm during the 8th sec of the experiment. In other words, at this point the average pupil dilation of the treatment group was 0.31 mm less than their response at the initial recording for the 8th sec. The response of the 10 treatment subjects demonstrated less dilation between 3 and 14 sec than did the placebo group. The 10th sec of the treatment group appeared to be an anomaly of the response pattern, although the response remained below the dilation recorded during the initial period.

The data were subjected to a statistical analysis of variance and covariance (22). The correct number of responses was input as the covariate, which removed the effect of the number of correct responses *before* performing an analysis of variance on the pupillary measures.

Table II displays the results of the analysis between the placebo and the drug treatment groups at the 11-sec point of the seven-digit, 2-hr task. The correct number of responses was modestly significant, and its effects were removed before significant differences of pupillary dilation between the treatment and control groups were detected. Table III displays the significance level of the removed covariate and the treatment effect at other seconds of the seven-digit, 2-hr task. The midportion of the experiment proved most significant for this task. By comparison, the nine-digit task at 2 hr did not generate statistically significant differences between the treatment and placebo groups.

DISCUSSION

The lack of difference in dilation at the nine-digit level is not surprising. In an experiment that employed random-digit recall tasks similar to those employed in this experiment, subjects began to overload during the 8th sec of a nine- and 13-digit memory task (9). When overloaded, the pupil

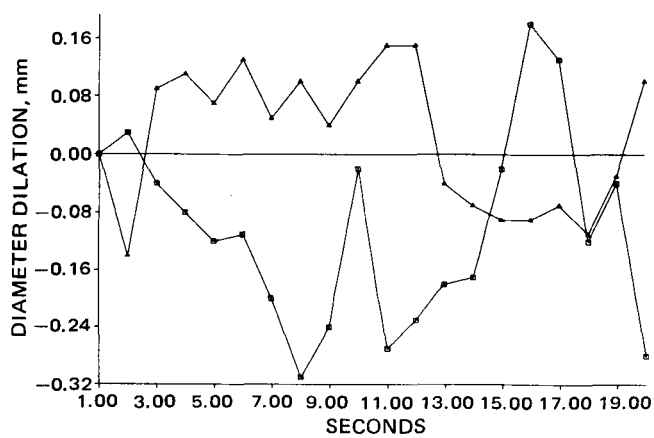

Figure 2—*Mean differential pupillary response of* T_0 *and* T_2 *hr for diazepam subjects (□) and placebo subjects (▲).*

Table III—Significance Level of Treatment and Covariate on Pupil Dilation for the Seven-Digit Task at 2 hr

Seconds	Covariate: Number Correct Responses	Main Effect: Drug Treatment
6	0.090	NS[a]
7	0.028	NS
8	0.160	0.263
9	0.142	NS
10	NS	NS
11	0.280	0.041
12	0.174	0.131
13	NS	NS

[a] NS = not significant.

diameter tended to level. Thus, the lack of significance during the nine-digit task of this experiment was attributed to processing overload. The subjects of both the control and treatment groups began to overload during the 11th sec of the experiment, which rendered further pupillary dilation impossible (23).

The maximum difference between the control and placebo groups was observed at the 11th sec, immediately following the recitation of the last digit. Kahneman *et al.* (24) demonstrated that pupils may continue to dilate following random-digit memory tasks as subjects continue to work on the regrouping of stored information. This fact was substantiated by Peavler (9), who demonstrated that maximum dilation occurred 1–2 sec following stimulation. Therefore, the most significant differential measures were observed at the point where maximum dilation occurred without overload. The differences quickly vanished following the 12th sec, representing a lessening of cognition activity and pupillary dilation.

Variability in measurements was expected. The effects of hippus, eyelid closure, fatigue, and other sources contributed to the variance. Also, blood levels of diazepam are considerably variable at 1 and 2 hr following an oral dose (16). Furthermore, the experiment did not include controls for anxiety levels, which contribute to the response to diazepam (25). Future applications of the cognitive task technique may enhance sensitivity if blood levels and psychological state are evaluated.

The results supported the hypotheses. The group treated with diazepam did not dilate to the degree of the placebo group in response to the seven-digit cognitive task. Additionally, drug administration reduced the ability of the subjects to recall the seven randomized digits.

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Impurities in Drugs II: Meperidine and Its Formulations

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Received June 21, 1977, from the *Drug Research Laboratories, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada.* Accepted for publication November 3, 1977.

Abstract □ Three lots of meperidine hydrochloride, seven lots of meperidine tablets, and 41 lots of meperidine injectables were examined for impurities by TLC. Impurities found were ethyl 1-benzyl-4-phenyl-4-piperidinecarboxylate, methyl 1-methyl-4-phenyl-4-piperidinecarboxylate, ethyl 1-ethyl-4-phenyl-4-piperidinecarboxylate, and three unidentified compounds. Not all impurities were found in every lot of drug investigated, and none of the impurities exceeded a concentration of 1% of the meperidine present.

Keyphrases □ Meperidine—TLC analyses of impurities in bulk drug and dosage forms □ TLC—analyses, impurities in meperidine bulk drug and dosage forms □ Impurities—in meperidine bulk drug and dosage forms, TLC analyses □ Narcotic analgesics—meperidine, TLC analyses of impurities in bulk drug and dosage forms

Impurities in drugs and their formulations may originate as intermediates or by-products during synthesis of the drug substance or as products of degradation during formulation or storage of the finished product, or they may result from drug–excipient interactions. To obtain adequate information on the number and level of impurities

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